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room is divided into two apartments connected with double doors, one of which is provided with hot water pipes.

"With these arrangements it was easy to keep the temperature within one or two degrees, above or below, that of the outer air, whatever the weather might be. The interior is furnished with tables in benches, work-tables, etc., for breeding-cage and root-cage work, and contains a brick-lined trench, three feet wide and fifteen feet long by three feet deep, cemented within, and filled with earth, for the larger plants, and for small plot experiments."

MICROSCOPY.¹

Notes on Celloidin Technique.—The high value of celloidin as an imbedding mass is well-known, and its superiority over all methods requiring heat is unquestioned, yet, from the fact that its manipulation has been attended by many difficulties, it has not come into general use. During the past two years I have tried the methods recommended by various authors and have found none entirely satisfactory, especially where very long series were necessary. The results of my experience are embodied in the following method:

The prepared plates or fragments are placed in an air-tight chamber; a four-ounce salt-mouth bottle being very suitable for this purpose. Pour into this bottle just enough ether-alcohol (equal parts acid-free sulphuric ether and absolute alcohol) to cover the fragments. The ether-alcohol should be added until after occasional shaking no celloidin remains undissolved; this may take several days. It should finally possess the consistency of a very thick oil. The solution thus obtained may be labelled No. 4. No. 3 is obtained by taking two volumes of No. 4 and diluting with one volume of ether-alcohol. No. 2 by proceeding in a like manner with No. 3. No. 1 is a mixture of absolute alcohol and sulphuric ether in equal parts.

The saturation and final imbedding is accomplished thus: The object is transferred from 95% alcohol to solutions 1, 2, 3, 4 successively, in each of which it remains from a few hours to days, depending upon the size and permeability. For pieces of tissue 2 mm. in diameter twenty-four hours in each will generally suffice. For a large brain, *e. g.* that of a cat, a week in each will not be too long.

In imbedding, unless orientation is desired, the ordinary paper box is best. A thin plate of lead is placed in the bottom and the imbed-

¹Edited by C. O. Whitman, Clark University, Worcester, Mass.

ding solution poured in. The object is taken from the same solution and with needles wet in ether placed in the desired position. Fine needles may be passed through the box to support the object.

In hardening, the method given by Viallanes of immersing in chloroform is preferable, since the operations may be carried on with much greater rapidity. An air-tight chamber should be filled with chloroform; a very wide-mouth bottle will answer. After the mass is thoroughly hardened, which requires about twenty-four hours, it is removed, the paper cut from the sides and transferred to 70% alcohol for a few hours.

It is now ready to fix for sectioning. Blocks are trimmed to fit the clamp of the microtome. Solution No. 3 is poured over the block; into this the celloidin block is pressed, after dipping the under surface in solution No. 1. Place in chloroform until hardened.

Reconstruction points are often very desirable. For this purpose the ordinary metallic imbedding box (fig. 1) made of two L-shaped pieces, *a* and *a'*, held in place by the overlapping strips *b*, is used. The ends and sides are perforated in as many places as desired by a very small drill. The holes should be so drilled that the silk threads which are drawn through run parallel. After being drawn tightly they are cemented to the sides of

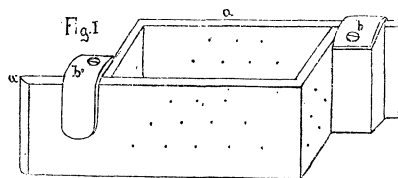


FIG. 1.

the box by a drop of celloidin. Five or six cm. of the thread should be left hanging. The bottom of the box is made by fitting in a piece of heavy blotting paper. The object is placed upon the threads in the desired position, and the imbedding mass poured in. As soon as hardened the celloidin holding the threads is dissolved by a drop of ether. The loose ends are soaked in solution No. 2, which has been thickened by the addition of lampblack. The threads are then drawn through, leaving the lampblack adhering to the celloidin, thereby forming excellent reconstruction points.

For small objects where reconstruction points are not needed the following method may be advantageously employed. The heads are clipped from fine insect pins, which are then placed in handles in such a way that they may be easily removed. On these pins the objects are oriented in the desired position; the pins are then removed from the handles and fixed in a cork (fig. II, *a*) previously perforated by a

somewhat larger pin. As fast as the pins carrying the objects are inserted the cork is replaced in the tube, which is filled with alcohol. A half dozen fish or amphibian ova may be oriented on the same cork. If desirable to draw the objects *in situ* a piece of lead may be pinned to the cork and the whole immersed in a small beaker of alcohol. The corks carrying the oriented objects are transferred successively to tubes containing the different solutions. When ready for final imbedding a piece of porous paper is wrapped about the tubes and cork and pinned. The cork is now removed, allowing the imbedding solution to fill the paper tube thus formed. A lead is fastened to the cork and the whole placed in chloroform until hardened, after which the paper is cut from the mass and the pins drawn through the cork, when it is ready for sectioning. This method offers many advantages in that several objects may be cut at the same time, drawings may be made after orientation, the objects are transferred from one solution to another more rapidly, etc.

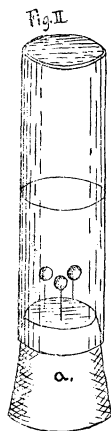


FIG. II.

In cutting, care should be taken that the knife is placed as obliquely as possible and kept constantly wet with 70% alcohol. For this purpose an ordinary pipette provided with a large rubber bulb is used. As fast as cut the sections are drawn back on the blade of the knife, by means of a needle, and arranged in a single row until the blade is filled. To remove them a heavy paper spatula is placed directly upon the section to which it adheres and may be drawn off the edge of the knife and transferred to the slide. By slight pressure together with a rolling movement the section is left in the desired position. Sufficient alcohol is kept on the slide to prevent drying but not enough to allow the sections to float. When the requisite number have been arranged they are covered with a strip of toilet paper which is held on the slide by winding it with fine thread. The sections being thus firmly held in position may be stained, etc. They should not be placed in absolute alcohol but cleared from 95% in a mixture of equal parts of bergamot oil, cedar oil and carbolic acid. When cleared the excess of fluid is removed by a piece of blotting paper; with gentle pressure sections which are by chance loose are firmly fixed in position, the thread is now cut, the strip of paper rolled back, balsam and cover applied.

If the object can be stained *in toto*, which is often the case, much time may be saved by the following method: The stained object is imbedded in the usual manner, but after hardening in chloroform, and

removing the paper, the celloidin block is transferred to 95% alcohol for twenty-four hours, then to carbolic acid¹ or glycerin in which it becomes as transparent as glass². The block is fixed in the usual manner.

Orientation is now accomplished with the greatest ease. In cutting, the knife is wet with the clearing medium given above. The sections may be arranged in serial order on the knife-blade until a slide-full is obtained, when they are transferred, balsam and cover applied. By this method long series may be readily handled. Glycerin is used only when the mounting medium is glycerin; in this case the knife is wet with glycerin.—A. C. EYCLESHYMER.

PROCEEDINGS OF SCIENTIFIC SOCIETIES.

Boston Society of Natural History.—April 6th.—The following papers were read: Percival Lowell, Shinto Occultism, Godpossession of the People; Harold C. Ernst, Some of the Advances in Bacteriology.—SAMUEL HENSHAW, *Secretary pro tempore*.

The Biological Society of Washington.—March 19th.—The principal paper of the evening was The Biological Basis of Psychology, by Prof. Lester F. Ward. The following communications were read: C. D. Walcott, On the Discovery of Certain Cambrian Fossils on the Coast of Massachusetts; F. H. Knowlton, The Fossil Flora of the Bozeman Coal Field; C. W. Stiles, Notes on Parasites. *Strongylus rubidus* Hassall and Stiles, '92.

April 2d.—The principal paper of the evening was The Interdependence of Plants and Insects, by Prof. C. V. Riley, illustrated by lantern slides. The following communications were made: C. Hart Merriam, The Distribution of Tree Yuccas, illustrated; H. E. Van Deman, Variations in the Fruit of *Hicoria Pecan*; C. W. Stiles, Notes on Parasites; Two Stages in the Life History of *Distoma magnum* Bassi 1875. (*D. americana* Hassall 1891.)—FREDERIC A. LUCAS, *Secretary*.

¹Bumpus, (Am. Nat., Jan. 1892) advises the use of thymol.

²Since discovering this method of rendering celloidin blocks transparent which was published in the Bot. Gaz. 1890 I have found that the clearing mixture given above answers the same purpose as the carbolic acid but requires a little longer time.